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Controlling Dahlia Powdery Mildew Disease Using Antagonistic Yeasts

Punlerdmatee, B., Cheewangkoon, R.* and To-anun, C.

Division of Plant Pathology, Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, 50200, Thailand.

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Abstract Powdery mildew caused by the fungus *Erysiphe cichoracearum* is a common disease of dahlia (*Dahlia pinnata* Cav.) grown in Thailand. This disease affects all parts of the plant and reduces plant quality. Yeasts antagonistic to *E. cichoracearum* were isolate from Thai fruits and vegetables. Four antagonists (CMY044, CMY019, CMY073 and CMY064) were found that inhibited *E. cichoracearum* growth with biocontrol efficacies of 100.00%, 88.85%, 88.85% and 86.68%, respectively. Yeast strain CMY044 showed efficacy in reducing the disease incidence of *E. cichoracearum* on dahlia leaves to as low as 0.00% compared to control plants (sterile distilled water). This result points to the potential applicability of naturally occurring yeast antagonists in an integrated disease management program for powdery mildew on dahlia. The research also included a study of *Ovulariopsis*/Painted spurge and the evaluation of other environmentally friendly fungicides.

Keywords: Biocontrol, Antagonist, Powdery mildew, *Erysiphe cichoracearum*

Introduction

Dahlia (*Dahlia pinnata* Cav.) is one of the most popular and attractive cut flower and garden plants around the world. Many specialists and consumers consider it the queen of summer flowers. Dahlia flowers can be exported as cut flowers where they have a long vase life and can also be used as pot or bedding plants for landscaping, especially in the form of single variety groups (Bradley, 1993).

Powdery mildew is one of the most common diseases of ornamental plants; many nursery, flower, and woody plants are susceptible Dicklow (2013). The fungi that cause powdery mildew all belong to the family, Erysiphaceae. Some powdery mildew fungi attack several different host plants, but most attack only a single host or, at most, only a few species. Most powdery mildew fungi produce a conspicuous white to grayish growth of fungal mycelium on the

* **Corresponding author:** Cheewangkoon, R.; **Email:** ratchadawan.c@cmu.ac.th

surface of the diseased plant part. Conidia, or spores of the fungus, are produced on the mycelium. In late summer most powdery mildew fungi also produce fruiting bodies called cleistothecia, which are dark-colored at maturity and about the size of coarse grains of pepper. These appear as dark specks on the white mycelium (Hanson, 2009).

Various management practices have been adopted to control this pathogen at farmers' fields. However, with the increase in awareness regarding the hazardous effects of synthetic fungicides, attempts are being made to go "back to nature" by managing the plant diseases with ecologically acceptable management practices. Among them, the use of botanicals has become an integral part of current research. Several plant products have been reported to be antifungal (Singh *et al*., 1980; Singh *et al*., 1990; Lyon *et al*., 1995; Suheyla *et al*., 1996) either by inducing resistance in hosts against pathogen attack or by directly inhibiting the growth of the pathogen.

Now, the use of many synthetic chemicals has been banned because of their potential toxic effect on the public health and their negative impact on the environment. So, implementing safe alternative methods (e.g. biological control) for controlling plant disease is becoming more urgent (Calvo, Calvente, Orellano, Benuzzi and Tosetti, 2007) .

Biological control using microbial antagonists has emerged as one of the most promising alternatives, either alone or as part of an integrated control strategy to reduce the input of synthetic fungicides (Mercier and Wilson, 1994; Chand-Goyal and Spotts, 1996; Benbowand Sugar, 1999; Fan and Tian, 2000). In the past 10 years, the interest in yeast antagonists has been increasing with the aim to isolate and test these species (*Candida saitoana, C. oleophila, C. sake, C. guillermondi, Debaryomyces hansenii, Metschnikowia fruticola, M. pulcherrima, Pichia anomala, Rhodotorula glutinis*, etc.) for their antimicrobial properties. (Csutak *et al*., 2013) The role of yeasts in biological control was recently been approved, because of their safety for public health (Droby *et al*., 2009).

The objectives of this study were to evaluate the potential of antagonistic yeasts as biological control agents to inhibit spore germination and control powdery mildew disease on dahlia under greenhouse conditions.

Material and Methods

Isolation and identification of the pathogen

Dahlia leaves showing symptoms of powdery mildew infection were collected from Bhubing Palace in Chiang Mai, Thailand. The pathogen was

identified primarily based on the morphological characteristics of the anamorph.

Yeast isolation and inoculum production

Yeast isolates were obtained from the epidermis of healthy leaves following the methodology described by Rabosto *et al*. (2006). Twenty leaves from each cluster were washed for 15 min with 250 mL of 0.1 mL tween 20 in 250 ml sterile distilled water. A 100 µL aliquot of the resulting solution was sown in Petri dishes containing yeast extract-peptone-dextrose medium (YEPD: 20 g dextrose, 20 g peptone, 10 g yeast extract, 20 g agar, and 1 L distilled water) supplemented with 0.05 g L-1 streptomycin (Sigma-Aldrich). These were incubated at 27 ºC until the development of the microorganism colonies in the culture medium was observed. Yeast colonies were isolated and cultured in YEPD medium. Finally, isolates were inoculated in tubes with inclined YEPD medium and stored at 4 ºC for subsequent analysis.

For inoculum production, yeasts were activated in 10 mL of yeast extract broth medium (3 g yeast extract, 5 g peptone per liter) in 250 mL flasks on a rotary shaker at 150 r/min at 27 ºC for 72 h. The yeast suspension was adjusted to a final concentration of 1×10^9 CFU/mL with a haemocytometer.

Laboratory experiments

Screening with *Ovulariopsis* sp. from Painted spurge on a thin layer of onion tissue

The 63 isolates of yeast were tested with *Ovulariopsis* sp. of Painted spurge on thin layer of onion tissue to select the potential antagonistic yeasts. According to the methods adopted by Nair and Ellingboe (1962), powdery mildew spores were harvested from only young leaves of Painted spurge. Yeast suspensions were prepared by subculture on PDA and the density was adjusted to 1 x 10^9 cells/mL^{-1} with the aid of a hemacytometer for use in inoculations. Yeast suspensions of the individual isolates were sprayed on thin layer of onion tissue (size: 1×1 cm^2). After that, new conidia of *Ovulariopsis* sp. were knocked onto the onion tissue; sterile distilled water served as the control. The inoculated cell layer of onion was floated on distilled water in a petri dish at room temperature for 24 h. Five replicates were used for each treatment, examined with a light microscope to observe conidial germination. The percentage of germinated spores was estimated according to the following formula:

$$\text{Percentage of germination} = \frac{\text{No. of germinated spores}}{\text{Total number of spores}} \times 100$$

Percentage of germination inhibition = 100 – Percentage of germination

Inhibitory effect of antagonistic yeast isolates on conidial germination of *E. cichoracearum* on PDA

A conidial inhibition assay was conducted to evaluate the antagonistic activity of yeast isolates against *E. cichoracearum* at room temperature according to the methodology described by Zhang *et al*. (2007), by selecting four of the most promising yeasts from the screening experiment with *Ovulariopsis* sp. from Painted spurge. A volume of 100 µL of the suspended yeast (1×10^9 cells/mL^{-1}) was spread on Petri dishes of potato dextrose agar (PDA) and then pathogen conidia were knocked onto the surface of the medium. The suspended yeast was replaced by 100 µL of sterile distilled water in the control treatment. Sulfur was also evaluated at 800 ppm (or 0.08% w/v). Spore germination was examined by observing 100 conidia of each treatment under a compound microscope. Conidial germination was observed and recorded at 3, 6, 9 and 12 h after application of the conidia.

The conidial germination inhibition index (GII %) was calculated according to the formula described by Manici *et al.* (1997):

$$\text{GII (\%)} = \frac{\text{conidia germinated in control} - \text{conidia germinated in treatment}}{\text{conidia germinated in control}} \times 100$$

Greenhouse experiments

Effect of antagonistic yeast isolates, and sulfur on *Erysiphe cichoracearum* powdery mildew severity in greenhouse

Greenhouse experiments were carried out during February – April 2016 at Bhubing Palace in Chiang Mai, Thailand. Plants were exposed to naturally occurring powdery mildew inoculum. Each treatment had four replications arranged in a randomized complete block design (RCBD)

Treatment	Fungicide
1	Control
2	Sulfur
3	CMY019
4	CMY044
5	CMY064
6	CMY073

For inoculum production, four of the yeasts were selected from the screen with *Ovulariopsis* sp. from Painted spurge on thin layer of onion tissue. Yeasts were activated in 10 mL of yeast extract broth medium (3 g yeast extract, 5 g

peptone per liter) in 250 mL flasks on a rotary shaker at 150 r/min at 27 ºC for 72 h. The yeast suspension was adjusted to a final concentration of 1×10^9 CFU/mL with a haemocytometer Sulfur treatment was evaluated at 800 ppm (or 0.08% w/v) and sterile distilled water served as the control. The suspensions were sprayed on 90 day-old dahlia test plants twice a week using a hand-sprayer.

The powdery mildew severity was recorded after spraying for 2 months (about 16 times) using a 0-10 scale modified from that described by Anonymous (2010) indicated in Table 1; Fig. 1 was also used as a guide.

Table 1. Powdery mildew severity rating scale (after Anon. 2010)

Powdery mildew rating	Percent disease severity
0	No symptoms of powdery mildew
1	1–10% of the leaf area infected
2	11–20% of the leaf area infected
3	21–30% of the leaf area infected
4	31–40% of the leaf area infected
5	41–50% of the leaf area infected
6	51–60% of the leaf area infected
7	61–70% of the leaf area infected
8	71–80% of the leaf area infected
9	81–90% of the leaf area infected
10	>90% of the leaf area infected

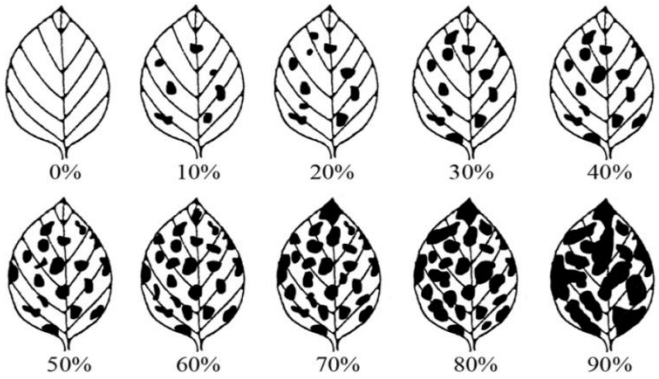

Figure 1. Rating of powdery mildew leaf symptoms (Vincelli and Hershman, 2011).

The severity of the disease was calculated using the following formula:

$$\text{Disease severity \%} = \frac{\Sigma (n \times v)}{5N} \times 100$$

Where:

n = Number of the infected leaves in each category.

v = Numerical values of each category.

N = Total number of the examined leaves.

The efficiency of the treatments was calculated according to the following formula:

$$\text{\% Efficiency} = \frac{\text{\% Infection in the control} - \text{\% Infection in the treatment}}{} \times 100$$

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and means were separated according to the least-significant-difference test (LSD) ($P \leq 0.05$).

Results

Symptoms and morphology characteristics of pathogen

The powdery mildew fungi were identified based on their anamorphic stage, the only stage present, using the general keys to species as described in the *Powdery Mildew Fungi* (Erysiphaceae) by To-anun and Takamatsu (2005).

Dahlia

Disease symptoms included grayish white circular to irregular patches consisting of epiphytic mycelia and conidia on stems, buds and leaves. As the disease progressed, leaves were covered by a gray powdery fungal mass, and older leaves became necrotic (Fig. 2).

The observations revealed the presence of epiphyllous mycelium, white, dense patches or covering the entire lower leaves surface; hyphal appressoria, nipple-shaped; Conidiophores were straight measured (10–)12.5–15 × (40–)87.5–135(–142.5) µm (average 13.46 × 110.38 µm), containing a mother cell forming conidia in chains, (5–)6.25–8.75(–10) × (30–)37.5–67.5 (–97.5) µm (average 7.38 × 54.71 µm); foot cell usually straight, (7.5–)10–12.5(-13.75) ×

(22.5–)42.5–65(–68.75) µm (average 11.25 × 53.83 µm); conidia were ellipsoid to ovoid, 12.5–17.5(–20) × (25–)27.5–32.5(–35) µm (average 15.79 × 29.58 µm); conidial germination formed fuliginea-type; present on the lateral side of the conidia. A perfect stage (chasmothecium) was not found. Powdery mildew in dahlia is caused by *Erysiphe cichoracearum*.

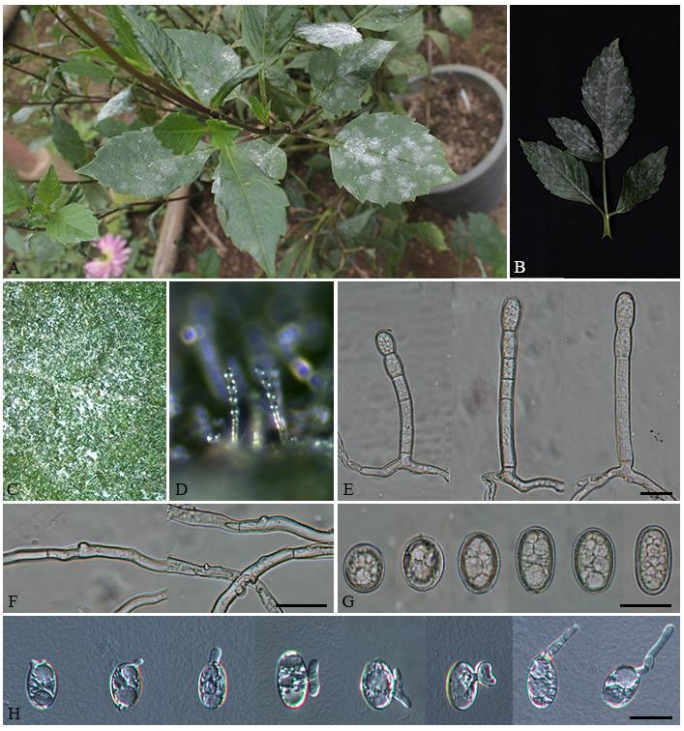

Figure 2. Morphology of Powderry Mildew on *Dahlia pinnata* Cav., family Asteraceae, A–B: Symptoms/signs of Powdery Mildew, C: Signs of Powdery Mildew under stereo microscope (10X), D: Signs of Powdery Mildew under stereo microscope (50X), E: Morphology of conidiophore–straight type, F: Morphology of mycelium and appressorium–nipple shaped, G: conidia–ellipsoid to ovoid and H: conidial germination fuliginea type (Scale bar= 20 µm).

Painted spurge

Typical symptoms include yellow- to brown-colored blotches on the upper surfaces of leaves and bracts; the white, powdery, dust-like structures that are a classic sign of the fungus typically appear first on the undersides of leaves (Fig. 3).

The observations revealed the presence of amphigenous mycelium, white, dense patches or covering the entire lower leaves surface; hyphal appressoria, lobe; Conidiophores were straight measured 6.25 – 7.50(–8.75) × (160.00–)180.00–275.00(–302.50) µm (average 7.17 × 227.33 µm), containing a mother cell, 3.75 – 6.25 × (50.00–)55.00 – 70.00 µm (average 5.13 × 63.33 µm); foot cell usually straight, 5.00 – 7.50 × 25.00 – 65.00(–70.00) µm (average 6.67 × 41.67 µm); conidiophore forming conidia 1 conidium per day, conidia were lanceolate, (12.50–)13.75 –21.25 × (47.50–) 55.00 – 72.50(–75.00) µm (average 16.21 × 64.46 µm); conidial germination formed polygoni type; present on the lateral side of the conidia. A perfect stage (chasmothecium) was not found. Powdery mildew in painted spurge is caused by *Ovulariopsis* sp.

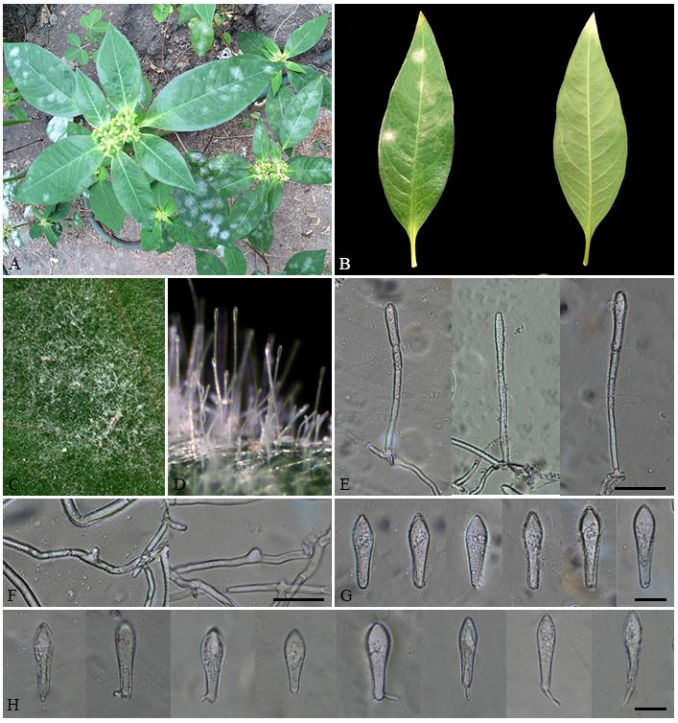

Figure 3. Morphology of Powdery Mildew of *Euphorbia heterophylla* L., Family Euphorbiaceae, A–B: Symptoms/signs of Powdery Mildew, C: Signs of Powdery Mildew under stereo microscope (10X), D: Signs of Powdery Mildew under stereo microscope (50X), E: Morphology of conidiophore–straight type, F: Morphology of mycelium and appressorium–lobed, G: conidia– single type and lanceolate shape and H: conidial germination - polygoni type (Scale bar= 20 µm).

Yeast isolation and selection

A total of 63 epiphytic yeasts were obtained. These were placed in the microorganism collection of the Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand and kept at 4^{o}C.

Laboratory experiments

Screening of yeasts against *Ovulariopsis* sp. from Painted spurge on a thin layer of onion tissue

Based on preliminary screening, 63 isolates of yeasts emerged as effective against *Ovulariopsis* on Painted spurge. Consequently, they were selected to study their efficacy in the inhibition of conidial germination of *Ovulariopsis* on Painted spurge. Table 2 shows that the percent of conidial germination was reduced by the yeasts with a low to high efficacy. Three yeasts (CMY044, CMY019 and CMY073) reduced conidial germination by 81.81%, 80.00% and 79.31% respectively.

Table 2. Effect of yeasts on inhibition of conidial germination of *Ovulariopsis* sp. from Painted spurge on thin layer of onion tissue

No.	Isolate of yeast	% inhibition of conidial germination	No.	Isolate of yeast	% inhibition of conidial germination
1	CMY044	81.81^{A}	33	CMY057	31.67^{LMN}
2	CMY019	80.00^{A}	34	CMY013	30.90^{LMNO}
3	CMY073	79.31^{A}	35	CMY031	30.30^{MNO}
4	CMY094	75.86^{A}	36	CMY147	29.30^{MNO}
5	CMY129	75.86^{A}	37	CMY096	29.08^{MNO}
6	CMY036	62.12^{B}	38	CMY058	28.79^{MNO}
7	CMY040	61.81^{B}	39	CMY150	28.79^{MNO}
8	CMY115	58.61^{BC}	40	CMY130	28.79^{MNO}
9	CMY102	58.18^{BCD}	41	CMY149	27.57^{MNO}
10	CMY064	52.72^{CDE}	42	CMY056	27.27^{MNOP}
11	CMY043	51.72^{CDEF}	43	CMY021	26.67^{NOP}

12	CMY029	51.52^{CDEF}	44	CMY045	24.24^{OPQ}
13	CMY006	50.90^{DEFG}	45	CMY005	19.98^{PQR}
14	CMY083	49.99^{EFGH}	46	CMY028	18.33^{QR}

Table 2. (Con.)

No.	Isolate of yeast	% inhibition of conidial germination	No.	Isolate of yeast	% inhibition of conidial germination
15	CMY059	49.99^{EFGH}	47	CMY120	18.18^{QRS}
16	CMY133	49.08^{EFGH}	48	CMY069	15.15^{RST}
17	CMY095	49.08^{EFGH}	49	CMY112	14.53^{RST}
18	CMY023	49.08^{EFGH}	50	CMY007	13.33^{RSTU}
19	CMY144	48.48^{EFGHI}	51	CMY136	13.33^{RSTU}
20	CMY014	48.33^{EFGHI}	52	CMY146	12.71^{RSTUV}
21	CMY077	44.82^{FGHIJ}	53	CMY028	10.89^{STUVW}
22	CMY074	43.63^{GHIJ}	54	CMY055	10.00^{TUVWX}
23	CMY142	43.62^{GHIJ}	55	CMY125	10.00^{TUVWX}
24	CMY151	43.09^{HIJ}	56	CMY076	9.09^{TUVWX}
25	CMY109	43.09^{HIJ}	57	CMY008	6.06^{UVWXY}
26	CMY070	43.09^{HIJ}	58	CMY038	5.44^{VWXY}
27	CMY081	43.09^{HIJ}	59	CMY107	4.55^{WXY}
28	CMY041	41.67^{IJK}	60	CMY015	4.55^{WXY}
29	CMY089	40.91^{JK}	61	CMY037	3.03^{XY}
30	CMY119	39.39^{JK}	62	CMY030	1.67^{Y}
31	CMY061	38.17^{JKL}	63	CMY040	1.52^{Y}
32	CMY025	34.53^{KLM}	64	Control	0.00^{Y}
CV%			13.10		
$LSD_{p\leq 0.05}$			7.34		

[1]The average was calculated using data from five replications.
[2]Values in the same column with different superscripts significantly differed by LSD at $P \leq 0.05$.

Inhibitory effect of antagonistic yeast isolates, and sulfur on conidial germination of *Erysiphe cichoracearum* on dahlia on PDA at 0, 3, 6, 9 and 12 hours

Table 3 indicated the percent inhibition of conidial germination compared with the control treatment (sterile distilled water). The data shows the efficiency of sulfur and the four yeast isolates. The highest percent inhibition of conidial germination (100%) was produced by sulfur and CMY044 at each observation time. Treatment with CMY073 reduced conidial germination at 3, 6, 9 and 12 h by 100%, 93.06%, 78.75% and 76.54%, respectively, while treatment with CMY064 reduced conidial germination at 3, 6, 9 and 12 h by 60.31%, 58.33%, 57.51% and 50.62%, respectively. The lowest inhibition of conidial germination was produced by CMY019: 57.14%, 51.39%, 43.76% and 39.50%, at 3, 6, 9 and 12 h, respectively.

Table 3. Effect of antagonistic yeasts, and sulfur on inhibition of conidial germination of *Erysiphe cichoracearum* on Dahlia on PDA at 0, 3, 6, 9 and 12 hours

Treatment	% inhibition of conidial germination				
	0	**3h**	**6h**	**9h**	**12h**
Control	0	0.00^{C}	0.00^{E}	0.00^{E}	0.00^{E}
Sulfur	0	100.00^{A}	100.00^{A}	100.00^{A}	100.00^{A}
CMY019	0	57.14^{B}	51.39^{D}	43.76^{D}	39.50^{D}
CMY044	0	100.00^{A}	100.00^{A}	100.00^{A}	100.00^{A}
CMY064	0	60.31^{B}	58.33^{C}	57.51^{C}	50.62^{C}
CMY073	0	100.00^{A}	93.06^{B}	78.75^{B}	76.54^{B}
CV%	-	2.81	3.58	6.12	5.98
$LSD_{p\leq 0.05}$	-	3.64	4.50	6.57	6.71

[1]The average of one hundred conidial observations for each treatment.
[2]Means followed by the same letter are not significantly different as determined by LSD, $P \leq 0.05$.

Greenhouse experiments

Effect of antagonistic yeast isolates and sulfur on *Erysiphe cichoracearum* powdery mildew severity in greenhouse

Plants treated with sulfur (30g/20L), and the four yeast isolates caused a significant reduction ($P<0.05$) in the development of powdery mildew from natural inoculum when compared to control plants.

The data in Table 4 shows the efficiency of all treatments in protection of dahlia leaves against the powdery mildew disease. However, CMY044 was the most effective isolate and reduced disease severity to 0.00%, as did the sulfur treatment. The treatment with CMY019 and CMY073 reduced the disease severity to 10.00%. CMY064 reduced the disease severity to 11.95%. The

characteristic disease symptoms observed with each treatment were different compared with the control. A high powdery mildew severity (89.7%) was observed in the control dahlia plants (Table 4; Fig.4 and 5). CMY044 and sulfur reduced disease severity to 0.0%, while the other yeasts were slightly less effective. Examination under the compound microscope showed that the most effective reduction of conidial production (0.0%) by *E. cichoracearum* was caused by CMY044 and sulfur compared to the control and CMY019, CMY064 and CMY073. The sulfur treatment completely inhibited fungal growth. (Fig.6).

Table 4. Effect of spraying antagonistic yeast isolates and sulfur on the severity of powdery mildew caused by *Erysiphe cichoracearum* in the greenhouse

Treatments	**Disease Severity %**	**Efficiency %**
Control (sterile distilled water)	89.72A	0.00F
Sulfur	0.00E	100.00A
CMY019	10.00D	88.85B
CMY044	0.00E	100.00A
CMY064	11.95C	86.68C
CMY073	10.00D	88.85B
LSD (0.05)	6.87	1.60
CV (%)	1.63	1.46

[1]The average was calculated using data from six observations on each of four replications for each treatment.
[2]Means followed by the same letter are not significantly different as determined by LSD, $P \leq 0.05$

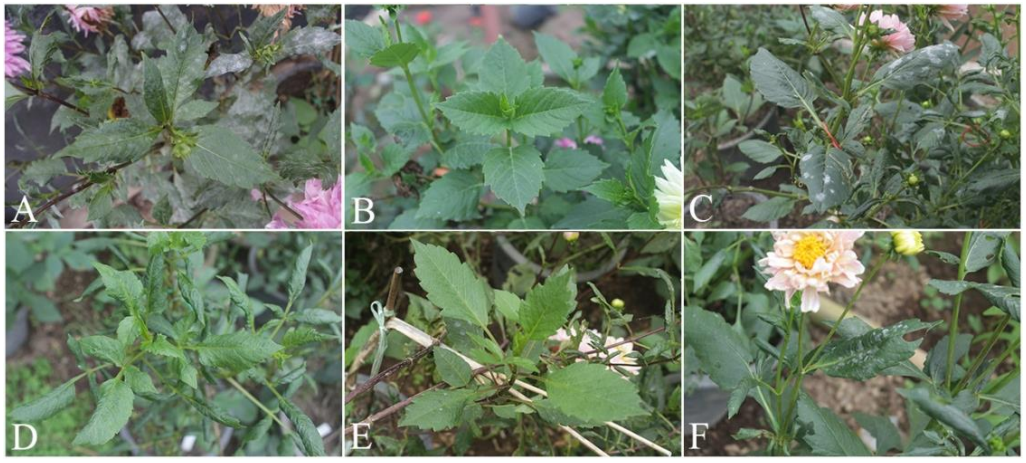

Figure 4. Effect of antagonistic yeast isolates and sulfur on disease severity (%) of powdery mildew of dahlia plants, A: control treated with sterile distilled water, B: sulfur, C: CMY019, D: CMY044, E: CMY064 and F: CMY073.

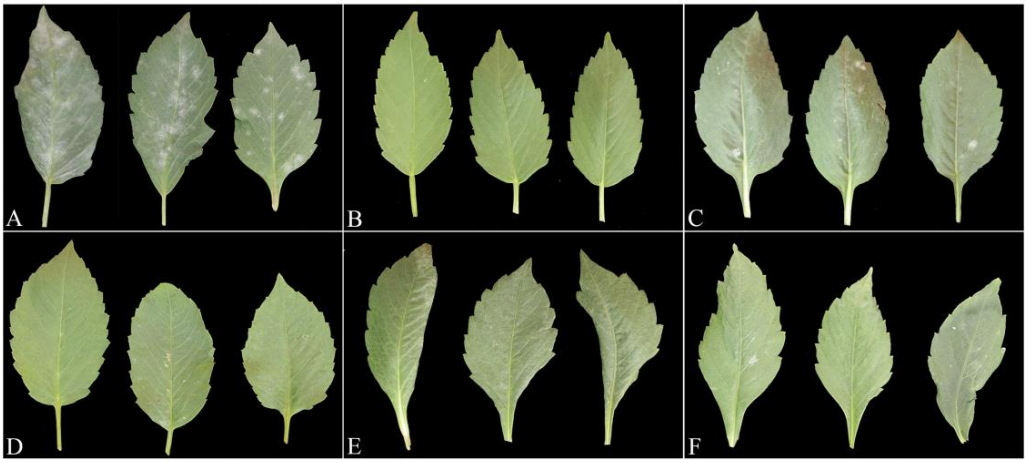

Figure 5. Effect of antagonistic yeast isolates and sulfur on disease severity (%) of dahlia leaves, A: control treated with sterile distilled water, B: sulfur, C: CMY019, D: CMY044, E: CMY064 and F: CMY073.

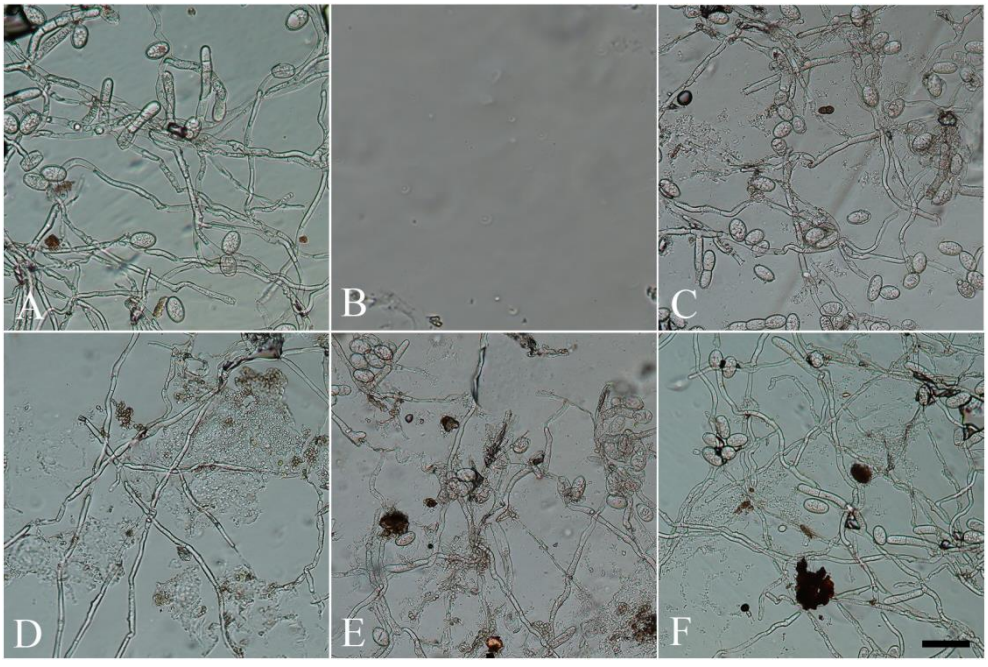

Figure 6. Effect of antagonistic yeast isolates and sulfur on disease severity (%) of powdery mildew of dahlia leaves under a compound microscope, A: control treated with sterile distilled water, B: sulfur, C: CMY019, D: CMY044, E: CMY064 and F: CMY073.

Discussion

On dahlia, powdery mildew disease caused by *Erysiphe cichoracearum* (synonym *Golovinomyces cichoracearum*) was identified based on morphology with the help of literature (Braun, 1987; Belanger *et al*., 2002). Biological control of different plant diseases has been focused on using bacteria or filamentous fungi (Whipps, 2001). So, application of yeasts as biocontrol agents represents a new trend against plant pathogens. In the last few decades this approach has become a positive alternative to chemical pesticides which is safe for humans, animals and the environment (Attyia and Youssry, 2001).

The laboratory experiments demonstrated the inhibitory effect of antagonistic yeast isolates on conidial germination of *E. cichoracearum* on PDA. The percentage of spore germination inhibition of CMY044 was the most effective at 100% compared to the control treatment (sterile distilled water) at 3, 6, 9 and 12 h in the spore germination experiment, and suggested that competition for nutrients by the yeast strains could have been one of the modes of action, which agrees with similar studies by Janisiewicz *et al.* (2000) and Grebenisan *et al*. (2008). Another mechanism could be parasitism and/or production of enzymes such as glucanases that degrade the pathogen cell wall; these are responsible for the degradation of cellulose and hemicellulose that are polymers making up the conidial walls (Masih and Paul, 2002). The application of different yeast antagonists such as *Debaryomyces hansenii* (Droby *et al*., 1989), *Pichia guilliermondii* (Arras *et al*., 1998) and *Aureobasidium pullulans* (Janisiewicz *et al*., 2000; Castoria *et al*., 2001) against *Penicillium* spp. gave similar results.

Disease symptoms appeared almost a month after transplanting and the powdery mildew epidemic developed slowly thereafter. During 4 weeks of the experiment, the relative humidity (RH) was below 80% and since powdery mildew develops best at a higher RH (80% to 90%) (Daughtrey *et al*., 1995), the low RH was probably a constraint to a faster epidemic development. This adverse microclimatic condition (low humidity) was probably useful for the plant cells that were already infected by the powdery mildew fungi in that it reduced the speed of the infection process giving the plant more time to transport material to the infection site and stop penetration by formation of papillae (Aust and Hoyningen-Huene, 1986). In this study under greenhouse

conditions, CMY044 was effective in reducing powdery mildew of dahlia to 100% compared with control plants (sterile distilled water). However, biocontrol of microorganisms can inhibit the growth of the infecting fungi without reducing the metabolic activity of the active hyphae. Competition among microorganisms for essential environmental factors, such as nutrients and space, is expected to have a dramatic effect on the secondary metabolism of spoilage moulds. In particular, nutritional competition has been reported to play a fundamental role in yeast–mould interactions (Chalutz *et al*., 1988; Bjornberg and Schnürer, 1993; Chand-Goyal and Spotts, 1996). Several antagonistic yeasts have previously been isolated from fruits and vegetables and efficaciously used as biocontrol agents. *Issatchenkia orientalis* strains 16C2 and 2C2 isolated from grape berry (Vitis vinifera L. cv. Negroamaro) were effective in reducing colonization of *Aspergillus carbonarius* (Bainer) Thom. and *Aspergillus niger* Tiegh. on grape berry (Bleve *et al*., 2006). *Colletotrichum musae* isolate 18 has been shown to have biocontrol efficacy against *Botrytis cinerea* Pers.: Fr. infection in strawberries (Fragaria x ananassa Duch. cv. Sweet Charlie) (El-Neshawy and Shetaia, 2003). Several strains of *P. guilliermondii* have biocontrol efficacy against infection by various fungi on citrus, apple, pear, table grape and strawberry (Arras *et al*., 1999; Droby *et al*., 1997; Lima *et al*., 1999). However, there has been no report on the use of yeasts to control *Erysiphe cichoracearum* on dahlia leaves. This study presents the first evidence that yeast strain CMY044 can reduce disease incidence caused by *E. cichoracearum* to as low as 0.00%. Strain CMY044 controlled powdery mildew disease on dahlia leaves at a level equal to the standard fungicide sulfur. The results suggest that yeast strain CMY044 has a high potential to be a biocontrol agent against *E. cichoracearum* infection of dahlia leaves.

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